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BIONANOSCULP, an ongoing project in biotechnology applications for preventive conservation of outdoor sculptures

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Abstract.
The objective of this paper is the presentation of the research strategies adopted and results of the ongoing BIONANOSCULP research project that is aiming to develop solutions in the area of sustainable nanomaterials, which are non-invasive and high-performance in their preventive conservation approach.
An integrated methodology was designed as a holistic strategy to the characterisation of the microbiota present on the surface of public outdoor sculptures. Gathering objective data in the characterization of the surface microbiota of public outdoor sculptures is important, in order to design strategies for the preventive conservation of these objects that make use of biotechnology innovative coatings. Such is one of the objectives of the project BIONANOSCULP. Methodologies applied include conservation reports, surface sampling methodologies using gels, 3D modeling, SEM, flow cytometry and metagenomics.
The project is already significantly contributing to create a bridge between the experts from different areas: the skills of biotechnologists, microbiologists, materials scientists, art historians and conservators-restorers to assess the state of conservation, biodeterioration and biocontamination of a selected number of sculptures, and to design the appropriate materials to pursue preventive conservation through coatings with anti-microbial activities.

1. Introduction

Urban outdoor public sculptures are among the most vulnerable cultural objects that can be found in a modern city. Subjected to extreme atmospheric variations and pollution, the conservation of such objects is, most of times, problematic. The variety of materials and the chemical and physical alterations suffered, as well as a wide range of microbiological and biological organisms that thrive on their surface increases the complexity of conservation treatments, and even more, definition of preventive conservation actions.

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Conservation treatments for this type of object usually aim at eradicating the responsible biological agents, however there is the need to aim further than devising restorative treatments that must be repeated every few years. This project aims expanding the traditional boundaries of conservation science to emergent scientific areas such as biotechnology and nanotechnology, changing the preventive conservation paradigm, taking the concept beyond climate control.

There are already several research efforts and reviews on biodeterioration of art objects and many on the specific microorganisms that are responsible for that process in stone and metallic sculptures [1]. They usually reveal important relationships between specific microorganisms and materials, but the variety of materials employed (from stone, to metal and human-made such as concrete) makes generalizations difficult. Since these objects often represent the past and validate the present of the inhabitants of the city, materializing their common cultural base, it is very important to find innovative solutions for conservation problems that affect them.

The need for preventive conservation in such cases is self-explanatory as it is impossible to control the environment that surrounds these objects and the sources for biocontamination responsible for the colonization, most of the time visible as biological patinas that grow into mature ecosystems comprising a large variety of microorganisms, bacteria, fungi, microalgae, lichens and small plants.

The research approach is based the development of innovative materials for coatings whose effectiveness will be tested in a real context, a limited number of public outdoor sculptures with selected material specificities from the metropolitan region of Oporto. The specific aims of the project are: (a) assessment of the state of biodeterioration and conservation state of a selected number of sculptures; (b) design the appropriate materials to pursue preventive conservation thru coating with anti-microbial and/or anti-UV activities as well as consolidation properties; and (c) to test their effectiveness over time.

A partnership with the Division of Culture of Oporto City Council, the Contemporary Sculpture International Museum of Santo Tirso (MIEC, http://miec.cm-stirso.pt) and the Museum of the Faculty of Fine Arts of Oporto (MFBAUP, http://museu.fba.up.pt) allowed access to seven selected sculptures from many different materials and forming part of the cultural heritage of the metropolitan region.

In a second development phase of the project, new coatings will be tested for their effectiveness in preventing the growth of the different agents involved, and also for the requirements for applications in this area. This includes interaction with the object (in this case, the sculpture) from the physical, chemical and aesthetic point of view, as well as reversibility, durability, and applicability to different types of materials. The clear advantage over other products is the low level of toxicity in our material, due to the use of biocompatible biomaterials such as chitosan [2].

One of the challenges of BIONANOSCULP is the interaction between researchers from such different scientific backgrounds such as biotechnologists, microbiologists, materials scientists, art historians and conservators-restorers as the proposed project is by nature collaborative and inter-disciplinary. As a result, it also aims at improving the dialogue between research and cultural heritage stakeholders.

Furthermore, expected products and protocols developed could be adopted by Municipalities, cultural heritage stakeholders, conservation and restoration institutions and companies. The results might also in the future be applied to other cultural heritage objects and collections both in a national and international dimension.

2. Methodologies

2.1 Sculptures selection, description and conservation status evaluation

The seven sculptures represent an ensemble with the common feature of being urban outdoor sculptures of the Oporto metropolitan area.

An initial survey of the catalogued public outdoor stone and metal sculptures in the Metropolitan Area of Porto was performed. The evaluation of the sculptures to integrate the research plan was achieved by observation of the materials present in the sculptures, as well as by visual recognition to the naked eye of the microbial contamination and their general conservation state. The accessibility and surrounding environmental conditions were also evaluated and taken into account.

From all the sculptures analyzed, the following objects were selected to integrate the research project:

Stone- and modern materials-based sculptures: *Rosalía de Castro* (1951) by Salvador Barata Feyo, pink granite, in Praça da Galiza, Massarelos, Porto; *Sol, Lua e Vento* (1997) by Satoru Sato, grey granite, in MIEC; *Afonso de Albuquerque* (1930) by Diogo de Macedo, limestone, in Largo de D. João III, Lordelo do Ouro, Porto; *Movimento* (1994) by Augusto Jorge Ulisses, marble, in MFBAUP; *Repouso* (1953) by Gustavo Bastos, cement mortar, in MFBAUP; *O guardador do Sol* (1953) by José Rodrigues, bronze, in MFBAUP (Fig. 1.) and *Eu espero* (1999) by Fernanda Fragateiro, stainless steel, in MFBAUP.

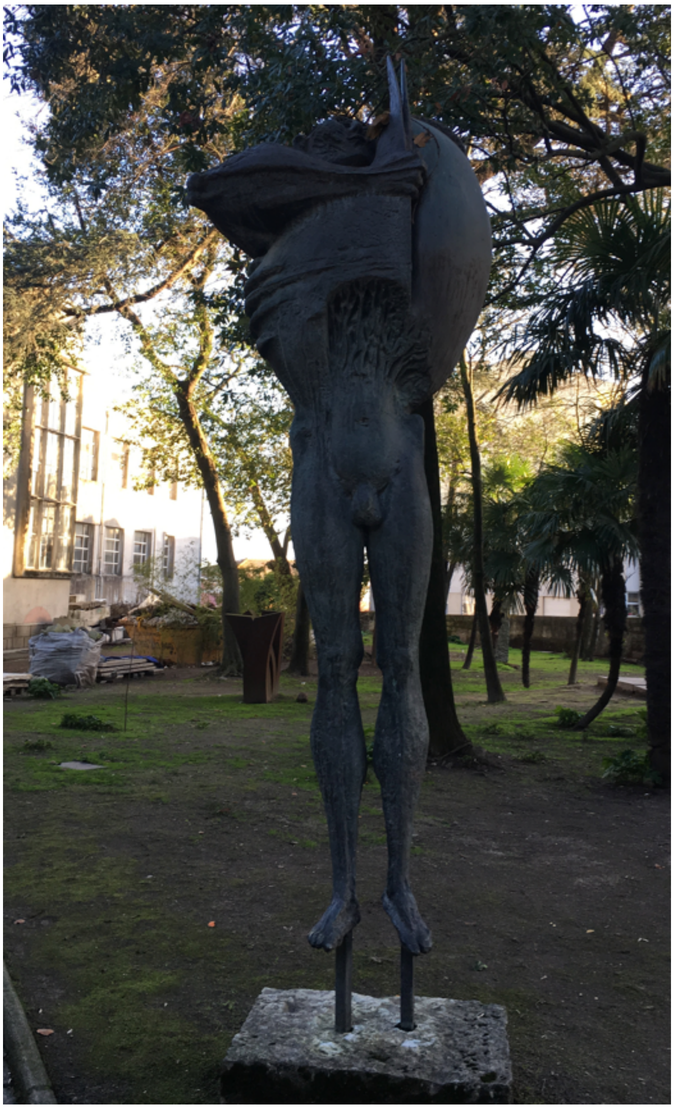

Figure 1. *O guardador do Sol* (1953) by José Rodrigues, bronze, in MFBAUP, one of the selected sculptures studied in BIONANOSCULP.

The selected sculptures are either constituted by stone or metal and all represent important Portuguese artists with the exception of *Sol, Lua e Vento*, a grey granite sculpture by Satoru Sato (MIEC). Their selection for the project was based not only on the materials present, easy access for sampling and (apparent) conservation state but also on their artistic and historical importance, that being reflected on the fact that two of the selected sculptures are also part of the Public Art Map rotes of the Oporto City Council that underlines the Public Art Program of the city (http://www.porto.pt/noticias/mapa-de-arte-publica-hoje-lancado-propoe-5-rotas-pelo-museu-a-ceu-aberto). The evaluation of conservation status was performed by the CITAR team researchers making use of an evaluation form specially designed since no previously defined form was available for outdoor sculptures. The art history specialist in the research team was able to gather important information about the artist, production details of the object, present and past locations, its relation to the institution that currently is responsible for its safeguard, as well as other relevant details that make up the history of the sculpture as detailed as can be described. Such information is often found disperse and is difficult to obtain but of incomparable importance for tracing the relationship between environment conditions and conservation status for outdoor objects.

2.2 3D Modeling and mapping analysis points

Nowadays it is also crucial to establish a knowledge database that includes 3D virtual models that can help explaining the objects [3]. These modeling process is important for mapping the state of biodeterioration and biocontamination, allowing the aquisition of details by the viewer that with a 2D image is not possible, namely in interfaces of materials and different surfaces. The data acquisition technique used is a type of photogrammetry, the structure from motion (SfM) technique. The photogrammetric 3D model was generated using Agisoft Photoscan® (http://www.agisoft.com/). This type of acquisition has the advantage of producing a photorealist 3D models of the sculptures. This characteristic is very important for mapping with accuracy the several locations where the nanomaterials will be tested. The photogrammetric models are then post-processed with Blender, a free and open source 3D creation suite software with GNU General Public License (www. blender.org). Figure 2 depicts a 3D model of *Repouso* (1953) by Gustavo Bastos.

Figure 2. 3D model of *Repouso* (1953) by Gustavo Bastos, in MFBAUP, one of the selected sculptures studied in BIONANOSCULP.

2.3 Surface sampling and characterization methodologies

After testing several gels including mixtures of PVA, HEMA, PVA/PVP and agar and agarose, a sampling procedure was defined that included swabbing with a sterile cotton swab and pressing a sterile disk of a HEMA/MBAm cryogel [4] onto the sculpture's surface followed by a protocol consisting of several centrifugations and filtrations to eliminate debris (adapted from [5]). Samples for SEM were collected with double-sided carbon adhesive tape and sputter-coated with gold and Fungi-tape™ (Scientific Device Laboratory, USA) was used to collect samples for optical microscopy at 100x amplifications. Two granite fragments were also collected from the sculpture with sterile tweezers and a chisel and used for flow cytometry, SEM and DNA extraction for metagenomics analysis. Figure 3 depicts an example of a SEM microcopy image obtained from sampling the surface of *Sol, Lua e Vento* sculpture in MIEC.

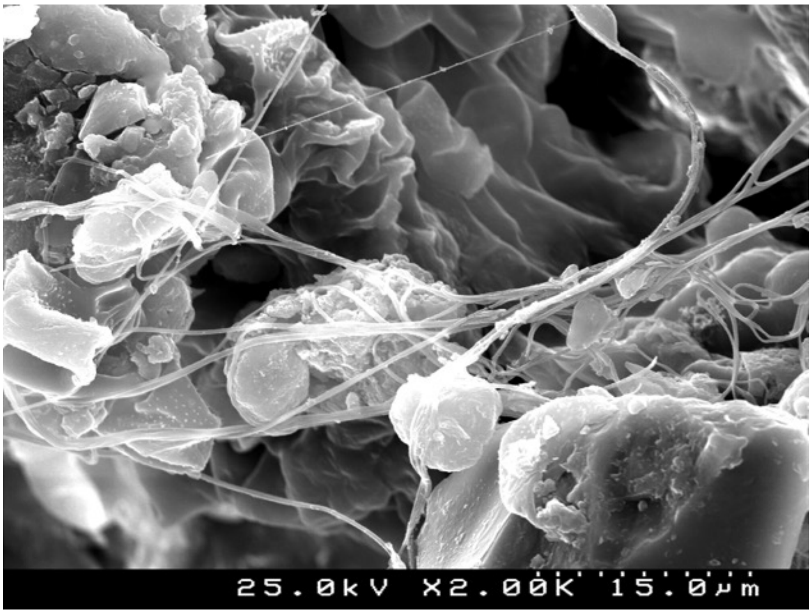

Figure 3. SEM image (2000x amplification) resulting of sampling the surface of *Sol, Lua e Vento* sculpture in MIEC, one of the selected sculptures studied in BIONANOSCULP.

2.4 Biocontamination quantification

Classic microbiology methodologies were performed from samples described in section 2.3 using Plate Count Agar (PCA) and Potato Dextrose Agar (PDA) by the spread plate method and incubated at 25 ˚C in the dark up to 7 days. For the growth of microalgae, BG-11 liquid media supplemented with amphotericin B was used. For aerobiology studies, PDA and PCA Petri dishes (in duplicate) were left open for 10 minutes in selected sites and incubated in the same conditions. Colorimetric measurements were performed using a CR-400 chroma meter (KONICA MINOLTA, Japan) on the sculpture’s surface in the five zones previously selected, and L*a*b* values were recorded. ATP was detected by swabbing the sculpture’s surface and determining the emitted light after reaction of the samples with a luciferin/luciferase mixture, using a HY-Lite®2 luminometer (Merck, Germany). BD Accuri™ C6 flow cytometer (BD Biosciences, USA) was used to acquired data without probes in order to calculate the number of autofluorescent microorganisms. Cell viability was determined after addition of thiazole orange (TO) and propidium iodide (PI) (BD™ Cell Viability Kit, BD Biosciences, USA).

2.5 Organism identification

To identify all culturable and unculturable microorganisms, total genomic DNA extraction was performed with the Ultraclean® Microbial DNA Isolation kit (MO BIO Laboratories, Inc., USA). DNA was quantified by spectrophotometry and amplified by PCR reaction using the bacterial and fungal universal primers 27F/1492R and ITS4/ITS5, for metagenomics analysis.

3. Discussion and conclusions

It was possible to devise a universal characterization protocol for outdoor sculptures that allows the integration of the results obtained by very different techniques, namely artistic and historical description, conservation reports, 3D modelling, surface characterization, biocontamination quantification and organism identification as described in sections 2.1 to 2.5. We believe this is the first time such a complete and interdisciplinary protocol is devised and performed for outdoor sculptures with the possibility to be applied independently of their material constitution, format, location and size. Gathering of data from the seven selected sculptures using previously described methodologies is still ongoing. The combination of the data obtained will allow the throughout characterization of the sculptures regarding their biocontamination and conservation status and surely allow for reaching insightful conclusions regarding their similarities and dissimilarities.

Within some of the methodologies, there were already interesting specific results, that result in innovation within the characterization of biocontamination. It was described for the first time, the use of a sampling hydrogel of HEMA/MBAm for cells and DNA in order do identify surface contamination on cultural objects.

It was also possible to evaluate the potential of using either swabs or hydrogels for samples that were later analysed by flow cytometry, which allowed for a quick and effective determination of the total number of microorganisms. Flow cytometry also allowed the determination of the total number of microorganisms that was confirmed by detection of ATP. Highest levels of ATP were observed in accordance with the results obtained with flow cytometry and growth on culture media.

The presence of autofluorescent microorganisms was detected, being higher in the samples collected with swabs than those collected with hydrogels. It was detected a great variation in the number of autofluorescent microorganisms detected, according to the methodology used to collect the samples (swab or hydrogel).

The development of the new coatings from chitosan is currently ongoing and in the near future they will be tested for their effectiveness in preventing the growth of the different biological agents identified on the sculptures; different requirements for application in preventive conservation will also be tested, including the coatings interactions with the object's materials from a physical, chemical, and aesthetic point of view, as well as reversibility, durability, and applicability to different types of materials. The clear advantage over other products is its low level of toxicity, which is very important from the perspective of the conservator-restorer or archaeologist.

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